from different perspectives to ensure at least one copy of every unique reporter is successfully discriminated.

One technique that might be used to read the reporter signature on beads using a conventional fluorescence microscopy apparatus would require that the beads be laid down on a planar substrate in order to present an optically readable bead array. If the beads were used in an assay prior to being affixed to a substrate, the assaying process should not disrupt the bound reporter signature. In the case where beads are affixed to a substrate before being used in an assay, the advantageous kinetics associated with the large surface area to volume ratio of free beads is lost. Nevertheless, the limitations of conventional microscopy techniques impose the requirement that beads be affixed to a substrate prior to analysis, thereby adding numerous preparatory steps to bead-based assays. These preparatory steps add time and expense, while simultaneously reducing the flexibility and utility of bead-based analytical processes.

In one bead arraying method, described in U.S. Patent No. 5,855,753, beads are placed on a substrate and caused to form a fixed monolayer through the use of an electric field. An "electrochemical sandwich" is formed by suspending the beads in an electrolytic fluid placed between an anode and a cathode. Using either an alternating current (AC) and or a direct current (DC) field applied to the sandwich in an appropriate manner, over time, the beads are caused to aggregate in specific groups, in a monolayer on the substrate.

Another bead arraying method is described in International Patent Application WO97/40385, which indicates that the electrochemical sandwich method is further enhanced by use of a specialized electrode in conjunction with externally applied illumination patterns that serve to further control the electrokinetic forces, which mediate bead aggregation on a substrate. U.S. Patent No. 5,695,934 discloses yet another method in which beads are laid on a substrate and affixed by chemical affinity between the functionalized surface of the substrate and bead-bound moieties. Other methods for arraying beads on substrates exist, but in all such methods, the goal is to ensure that the bead layer is fixed in place, preventing movement of the beads during the process of reading the beads. Typically, in any microscopy process for reading beads, it would be necessary to affix the bead-substrate to a two-axis stage and move the stage in a pattern that enables each portion of the substrate to be read. This process involves numerous cycles of acceleration and deceleration of the substrate as it is moved on the stage, which would likely induce independent movement of the beads, if they are not securely affixed to the substrate.

Bead movement is not the only complication associated with reading labeled beads on a planar substrate. Another consideration is the need for achieving an

accurate focus across the field of view (FOV), which can be compromised by any non-planarity of the packed beads on the substrate or by any non-planarity of the substrate itself. For these reasons, the focus on each portion of the bead array must be individually achieved to ensure proper resolution. Although autofocus systems are well known in the art, the focus step requires additional time, expense, and adds variability to the process. Additional images may be required to discriminate the different fluorescence emission spectra of bead-bound signaling molecules and of the reporters themselves. In addition, if the signaling molecules or reporters are randomly distributed on the beads, it may not be possible to identify signals or signatures from a fraction of the beads due to the absence of the signals or reporters from the planar FOV. The planar substrate preparation presents only one, or at most two, of six possible perspectives from which to view the bead, increasing the likelihood that reporters will be hidden from the imaging system.

The complexity associated with arraying beads hinders bead-based analytical approaches, regardless of the number of beads in the library. As the size of an analytical bead library grows beyond roughly a million beads, the substrate-based approach to bead imaging becomes highly impractical. Significant difficulties arise when tens of millions to billions of beads must be analyzed, requiring that the bead array substrates grow substantially in size. The difficulties involved in creating a uniform, tightly packed, fixed array increase greatly with the size of the array. Furthermore, accurate and rapid positioning of the array during the imaging process becomes far more difficult. The size, expense, and low throughput of such systems rule out their widespread use in research and for point-of-care applications. Therefore, an improved method for analyzing beads is desired. Preferably this method should eliminate the need for placing the beads on a substrate and enable the simultaneous imaging of multiple focal planes and multiple bead orientations. Ideally, this new method would simultaneously image the entire spectrum of bead fluorescent emissions and provide enough spectral resolution to discriminate the colors originating from each reporter. Finally, an ideal method would conveniently handle billions of beads and enable ultra-high speed imaging to analyze large bead libraries in a matter of hours.

Summary of the Invention

In accord with the present invention, a method and apparatus for imaging and reading reporter labeled beads are provided. The method and apparatus of the present invention enable individual encoded beads to be imaged, and the compound attached to that bead to be identified as a function of image data, so that the identity and sequence of all sub units of the compound are determined. Generally, a single encoded bead will include redundant copies of the same compound and the associated reporters. In one

preferred application of the present invention, the sub units are oligos, the resulting compound is an oligonucleotide, and each reporter is preferably discriminable by its color. However, other types of sub units forming other types of compounds and reporters that are uniquely discriminable by characteristics other than color can be imaged and decoded using the method and system of the present invention. Essentially, any characteristic that is determinable via imaging, such as size and shape, can be employed as a reporter characteristic.

It should also be understood that the method and apparatus of the present invention may be applied to image and decode reporter labeled beads independently of the process employed to produce such reporter labeled beads. It is anticipated that processes such as stochastic synthesis, directed synthesis, combinations thereof, or the attachment of a pre-synthesized compounds to previously (or subsequently) encoded reporter labeled beads will be employed to produce reporter labeled beads. Unlike a stochastic SAP process, in a directed synthesis the path of each bead is predetermined during compound synthesis and reporter addition. When reporter labeled beads are used with pre-synthesized compounds all unique reporter types may be bound to the carrier in one step to create a unique signature for the bead. In this case the unique combination of reporters bound to a bead simply create a unique identity and do not necessarily encode the subunit sequence for the compound bound to the bead. A cross reference table or other means may be created to correlate bead signature to compound identity. In this case the present invention may be used to identify the unique bead signature to determine compound identity by searching the cross reference table.

The basic method involves focussing light from an encoded bead along a collection path, and dispersing the light into a plurality of light beams as a function of a plurality of different discriminable characteristics of the light that are indicative of the plurality of different reporters associated with the encoded bead. Each light beam is focussed to produce a respective image corresponding to that light beam. A plurality of signals are generated in response to the respective images thus produced. Each signal generated indicates whether a different one of the plurality of reporters is associated with the encoded bead. The signals are analyzed to decode a sequence and to identify the sub units forming a component attached to the encoded bead, and these steps are repeated for successive encoded beads.

Preferably, the step of dispersing comprises the steps of dividing the light into the plurality of light beams as a function of the wavelength of the light beams. Also preferably, the step of analyzing the plurality of signals comprises the step of constructing a sequence library of the plurality of components based on each encoded bead that is decoded.